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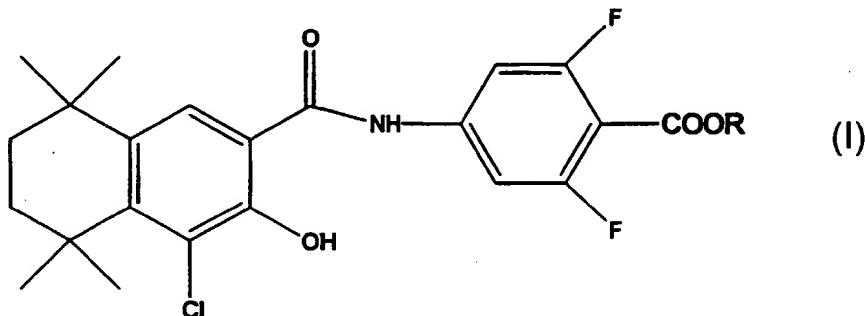
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(54) Title: TREATMENT OF TUMORS WITH RAR ALPHA SELECTIVE RETINOID COMPOUNDS IN COMBINATION WITH OTHER ANTI-TUMOR AGENTS



(57) Abstract: Compounds which are specific or selective agonists of RAR α receptors in preference over RAR β and RAR γ receptors, and particularly compounds of the formula (I) where R is a H, lower alkyl of 1 to 6 carbons, or a pharmaceutically acceptable salt, are useful for treating a malignant disease or condition in a mammal. In treatment of solid tumors the compound exhibit synergistic antiproliferative effect with human recombinant interferon.

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TREATMENT OF TUMORS WITH RAR ALPHA SELECTIVE RETINOID COMPOUNDS IN COMBINATION
WITH OTHER ANTI-TUMOR AGENTS

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BACKGROUND OF THE INVENTION

6 1. Field of the Invention

7 The present invention relates to the use of RAR α specific or selective
8 retinoid compounds in combination with interferons and other anti-tumor
9 agents. More particularly the present invention relates to the use of RAR α
10 specific or selective retinoid compounds for the treatment of carcinoma of the
11 breast in combination with interferons and other anti-tumor agents. Still more
12 particularly, the present invention relates to the use of 4-[(4-chloro-3-
13 hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-
14 amino]-2,6-difluoro-benzoic acid and related compounds in combination with
15 interferons and other anti-tumor agents, and specifically to the use of 4-[(4-
16 chloro-3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalene-2-
17 carbonyl)-amino]-2,6-difluoro-benzoic acid and related compounds for the
18 treatment of carcinoma of the breast in combination with interferons and other
19 anti-tumor agents.

20 2. Background Art

21 Naturally occurring retinoic acid and related compounds, generally
22 called retinoids, have been known in the biopharmaceutical, medical and
23 related arts to have of important biological activity, including prevention and
24 inhibition of malignant cell proliferation. A vast volume of patent and
25 scientific literature exists describing the synthesis of retinoid compounds,
26 their biological activities and investigations aimed at discovering the varying
27 modes of action of retinoids in human and other biological systems, *in*
28 *vitro* and *in vivo* as well.

1 Specifically, it is generally accepted in the art that in the anti-cell-
2 proliferative or anti-tumor field, pharmaceutical compositions having a
3 retinoid-like compound or compounds as the active ingredient are useful for
4 treating or preventing hyperproliferative disorders of the skin, and other
5 premalignant and malignant hyperproliferative diseases such as cancers of the
6 breast, skin, prostate, cervix, uterus, colon, bladder, esophagus, stomach, lung,
7 larynx, oral cavity, blood and lymphatic system, metaplasias, dysplasias,
8 neoplasias, leukoplakias and papillomas of the mucous membranes and in the
9 treatment of Kaposi's sarcoma. However, a generally recognized disadvantage
10 of treatment of mammals by retinoids is their mucocutaneous toxicity which
11 occurs in greater than 90% of patients when treated with an effective dose of
12 retinoids, topically or systemically.

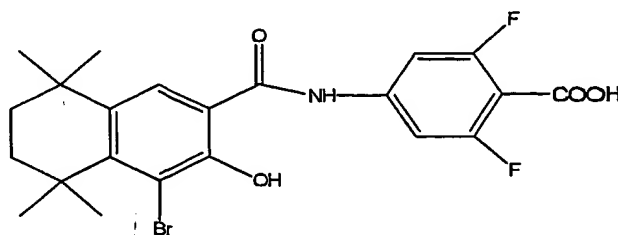
13 It is now also general knowledge in the art that two main types of
14 retinoid receptors exist in mammals (and other organisms). The two main
15 types or families of receptors are respectively designated the RARs and
16 RXRs. Within each type there are subtypes; in the RAR family the subtypes
17 are designated RAR α , RAR β and RAR γ , in RXR the subtypes are: RXR α ,
18 RXR β and RXR γ . It has also been established in the art that the distribution
19 of the two main retinoid receptor types, and of the several sub-types is not
20 uniform in the various tissues and organs of mammalian organisms.
21 Moreover, it is generally accepted in the art that many unwanted side effects
22 of retinoids, such as the mucocutaneous toxicity are mediated by one or more
23 of the RAR receptor subtypes. A publication by *Standeven et al.*, Toxicology
24 Letters 92 (1997) 231-240 discloses that treatment of mice by RAR α selective
25 retinoids results in significantly reduced skin irritation (mucocutaneous
26 toxicity) than treatment with retinoids which have strong RAR β and
27 particularly RAR γ agonist activity.

28 United States Patent No. 5,965,606 discloses methods of treatment of
29 tumors with RAR α specific or selective retinoids, and the synthesis of such

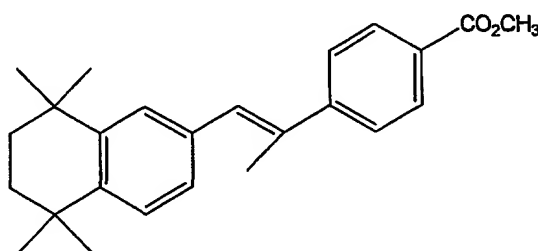
1 retinoids is described in this patent as well as in United States Patent No.
2 5,856,490. An important RAR α selective compound of United States Patent
3 No. 5,965,606 (Compound 32 of this patent reference) is shown below.

4 With regard to using retinoids in combination with other drugs to treat
5 tumors, there are published reports in the art that certain retinoid compounds
6 act additively and some even synergistically with other known anti-tumor
7 chemotherapeutic agents, such as interferons and other drugs, in several
8 carcinoma of the breast cell cultures to suppress or inhibit the proliferation of
9 the cancer cells. The publication by *Fanjul et al.* in Cancer Research **56**, 1571
10 - 1577 (1996) describes assays of several retinoid compounds, including a
11 compound designated in the publication as SRI 11220 in combination with
12 interferon in several carcinoma cell lines, and states that in some of the cell
13 lines the anti-proliferative activity of the compound SRI 11220 and interferon
14 was synergistic. The structure of this prior art compound SRI 11220 is shown
15 below. Significantly however, the *Fanjul et al.* reference attributes the
16 inhibition of breast cancer cells by selective retinoids and interferon to the
17 potential role of the RAR γ receptors. In fact, the compound SRI 11220 is
18 disclosed in this reference as an RAR γ agonist.

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Compound 32 of U.S. Patent No. 5,965,606
Compound 36 of U.S. Patent No. 5,856,490
Prior Art



SRI 11220 (Prior Art)

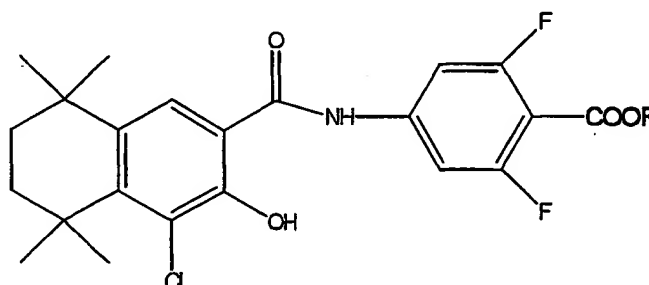
A publication by *Toma et al.* in International Journal of Oncology **10**: 597 - 607 (1997) describes synergistic effects of certain other retinoids, such as all trans retinoic acid (tRA) with α interferon (α IFN) and synergistic effect with other chemotherapeutic agents such as tamoxifen (TAM) in MCF-7 human breast cancer lines. As further background to the present invention it is noted that a publication by *Kurbacher et al.* in Cancer Letters **103** (1996) 183 - 189 describes synergistic action of vitamin C with certain chemotherapeutic anti-tumor agents in MCF-7 and MDA-MB 231 human carcinoma cell lines.

United States Patent No. 5,856,490 discloses aryl or heteroaryl amides of tetrahydronaphthalenes, which are generally speaking RAR α specific retinoids. Among the compounds specifically described as preferred embodiments in that reference is the 2,6-difluoro-4-[3'-hydroxy-4'-bromo-5',6',7',8'-tetrahydro-5',8',8'-tetramethylnaphthalen-2'-yl]carbamoyl]benzoic acid, the structure of which is shown above. In the 5,856,490 reference this compound is designated compound 36.

SUMMARY OF THE INVENTION

The present invention relates to the use of RAR α specific or selective retinoids in combination with other anti-tumor agents for the treatment of a malignant tumor or condition in a mammal in need of such treatment. The RAR α specific or selective retinoid is generally speaking administered to the mammal in need of such treatment in a pharmaceutical composition comprising a pharmaceutically acceptable excipient and the RAR α specific or selective retinoid as the active ingredient. The other anti-tumor agent of the combination therapy may be administered in the same or in a different pharmaceutical composition.

The present invention also relates to compounds of **Formula 1**



R = H or lower alkyl of 1 to 6 carbons

FORMULA 1

where R represents H or a lower alkyl group having 1 to 6 carbons, and to pharmaceutically acceptable salts of said compounds, and to the use of compounds of **Formula 1** in combination with other anti-tumor agents for the treatment of a malignant tumor or condition in a mammal in need of such treatment. Furthermore, the present invention also relates to a pharmaceutical composition for treatment of a malignant tumor or condition in a mammal in need of such treatment, where the active ingredient of the composition comprises one or more compounds of **Formula 1**. Such pharmaceutical

- 1 composition comprising as its active ingredient one or more compounds of
- 2 **Formula 1** is advantageously used in combination with one or more other
- 3 anti-tumor agents for the treatment of a malignant tumor or condition in a
- 4 mammal in need of such treatment.

1 BRIEF DESCRIPTION OF THE DRAWINGS

2 **Figure 1** is a graph showing synergism in the anti-proliferative effects
3 of a combination of the compound AGN 195183 (Compound 2) of the
4 invention and of α interferon (IFN α) in SKBR-3 cells.

5 **Figure 2** is a graph showing synergism in the anti-proliferative effects
6 of a combination of the compound AGN 195183 (Compound 2) of the
7 invention and of β interferon (IFN β) in SKBR-3 cells.

8 **Figure 3** is a graph showing synergism in the anti-proliferative effects
9 of a combination of the compound AGN 195183 (Compound 2) of the
10 invention and of γ interferon (IFN γ) in SKBR-3 cells.

11 **Figure 4** is another graph showing synergism in the anti-proliferative
12 effects of a combination of the compound AGN 195183 (Compound 2) of the
13 invention and of α interferon (IFN α) in SKBR-3 cells.

14 **Figure 5** is another graph showing synergism in the anti-proliferative
15 effects of a combination of the compound AGN 195183 (Compound 2) of the
16 invention and of β interferon (IFN β) in SKBR-3 cells.

17 **Figure 6** is another graph showing synergism in the anti-proliferative
18 effects of a combination of the compound AGN 195183 (Compound 2) of the
19 invention and of γ interferon (IFN γ) in SKBR-3 cells.

20 **Figure 7** is a graph showing the anti-proliferative effects of a
21 combination of the compound AGN 195183 (Compound 2) of the invention
22 and of α interferon (IFN α) in T47-D cells.

23 **Figure 8** is a graph showing synergism in the anti-proliferative effects
24 of a combination of the compound AGN 195183 (Compound 2) of the
25 invention and of β interferon (IFN β) in T47-D cells.

26 **Figure 9** is a graph showing synergism in the anti-proliferative effects
27 of a combination of the compound AGN 195183 (Compound 2) of the
28 invention and of γ interferon (IFN γ) in T47-D cells.

29 **Figure 10** is another graph showing the anti-proliferative effects of a

1 combination of the compound AGN 195183 (Compound 2) of the invention
2 and of α interferon (IFN α) in T47-D cells.

3 **Figure 11** is another graph showing synergism in the anti-proliferative
4 effects of a combination of the compound AGN 195183 (Compound 2) of the
5 invention and of β interferon (IFN β) in T47-D cells.

6 **Figure 12** is another graph showing synergism in the anti-proliferative
7 effects of a combination of the compound AGN 195183 (Compound 2) of the
8 invention and of γ interferon (IFN γ) in T47-D cells.

9 **Figure 13** is a graph showing the effect of compound AGN 195183
10 (Compound 2) of the invention in SKBR-3 and in T47-D cells.

11 **RAR α SPECIFIC OR SELECTIVE COMPOUNDS USED IN THE**
12 **INVENTION, ASSAYS TO ESTABLISH SELECTIVITY**

13 RAR α specific and or RAR α selective compounds can be obtained, for
14 example, as described in United States Patent Nos. 5,856,490 and 5,965,606,
15 the specifications of which are expressly incorporated herein by reference.
16 These references also present data to show that the compounds are indeed
17 RAR α specific or selective agonists. Assays by which a compound can be
18 tested and established whether or not it is an RAR α specific or selective
19 agonist, are known in the art and are described in numerous prior art
20 publications and patents. For example, a **chimeric receptor transactivation**
21 **assay** which tests for agonist-like activity in the RAR α , RAR β , RAR γ , RXR α
22 receptor subtypes, and which is based on work published by *Feigner P. L. and*
23 *Holm M.* (1989) Focus, 112 is described in detail in United States Patent No.
24 5,455,265. The specification of United States Patent No. 5,455,265 is
25 hereby expressly incorporated by reference.

26 **A holoreceptor transactivation assay and a ligand binding assay**
27 **which measure the antagonist/agonist like activity of the compounds of the**
28 **invention, or their ability to bind to the several retinoid receptor subtypes,**
29 **respectively, are described in published PCT Application No. WO**

1 WO93/11755 (particularly on pages 30 - 33 and 37 - 41) published on June
2 24, 1993, the specification of which is also incorporated herein by reference.
3 A description of the ligand binding assay is also provided below.

4 LIGAND BINDING ASSAY

5 All binding assays were performed in a similar fashion. All six
6 receptor types were derived from the expressed receptor type (RAR α , β , γ
7 and RXR α , β , γ) expressed in Baculovirus. Stock solutions of all compounds
8 were prepared as 10 mM ethanol solutions and serial dilutions carried out into
9 1:1 DMSO; ethanol. Assay buffers consisted of the following for all six
10 receptor assays: 8% glycerol. 120 mM KCl. 8 mM Tris. 5 mM CHAPS 4 mM
11 DTT and 0.24 mM PMSF. pH-7.4@ room temperature.

12 All receptor binding assays were performed in the same manner. The
13 final assay volume was 250 μ l and contained from 10-40 μ g of extract protein
14 depending on receptor being assayed along with 5 nM of [3 H] all-trans retinoic
15 acid or 10 nM [3 H] 9-cis retinoic acid and varying concentrations of competing
16 ligand at concentrations that ranged from 0-10⁻⁵M. The assays were formatted
17 for a 96 well minitube system. Incubations were carried out at 4° C. until
18 equilibrium was achieved. Non-specific binding was defined as that binding
19 remaining in the presence of 1000 nM of the appropriate unlabeled retinoic
20 acid isomer. At the end of the incubation period. 50 μ l of 6.25%
21 hydroxyapatite was added in the appropriate wash buffer. The wash buffer
22 consisted of 100 mM KCl. 10 mM Tris and either 5 mM CHAPS (RXR α , β ,
23 γ) or 0.5% Triton X-100 (RAR α , β , γ). The mixture was vortexed and
24 incubated for 10 minutes at 4° C., centrifuged and the supernatant removed.
25 The hydroxyapatite was washed three more times with the appropriate wash
26 buffer. The receptor-ligand complex was adsorbed by the hydroxyapatite. The
27 amount of receptor-ligand complex was determined by liquid scintillation
28 counting of hydroxyapatite pellet.

1 After correcting for non-specific binding, IC_{50} values were determined.
2 The IC_{50} value is defined as the concentration of competing ligand needed to
3 reduce specific binding by 50%. The IC_{50} value was determined graphically
4 from a loglogit plot of the data. The K_d values were determined by application
5 of the Cheng-Prusoff equation to the IC_{50} values, the labeled ligand
6 concentration and the K_d of the labeled ligand.

7 The results of **ligand binding assay** are expressed in K_d numbers.
8 (See *Cheng et al.* Biochemical Pharmacology Vol. 22 pp 3099-3108,
9 expressly incorporated herein by reference.)

10 A detailed experimental procedure for **holoreceptor transactivations**
11 has been described by *Heyman et al.* *Cell* 68, 397 - 406, (1992); *Allegretto et*
12 *al.* *J. Biol. Chem.* 268, 26625 - 26633, and *Mangelsdorf et al.* *The Retinoids:*
13 *Biology, Chemistry and Medicine*, pp 319 - 349, Raven Press Ltd., New York,
14 which are expressly incorporated herein by reference. The results obtained in
15 this assay are expressed in EC_{50} numbers, as they are also in the **chimeric**
16 **receptor transactivation assay**.

17 In the chimeric transactivation assay **Compound 2** of the present
18 disclosure was found to have an EC_{50} value of 180 nanomolar with 75 %
19 efficiency at the $RAR\alpha$ receptors, and in the ligand binding assay a K_d value
20 of 5 nmolar. For $RAR\beta$ and $RAR\gamma$ receptors **Compound 2** was found to be
21 inactive as an agonist, with an EC_{50} values greater than 10^4 nanomolar.

22 Still another transactivation assay, the "PGR assay" is described in the
23 publication *Klein et al.* *J. Biol. Chem.* 271, 22692-22696 (1996) which is
24 expressly incorporated herein by reference, and a detailed description is also
25 provided below. The results of the PGR assay are also expressed in EC_{50}
26 numbers (nanomolar concentration).

27 **RAR-P-GR Holoreceptor Transactivation Assay**

28 CV-1 cells (4×10^5 cells/well) were transiently transfected with the
29 luciferase reporter plasmid MTV-4(R5G)-Luc (0.7 ug/well) containing four

1 copies of the R5G retinoid DNA response element along with the RXR α
2 expression plasmid pRS-hRXR α (0.1 ug/well) and one of the RAR-P-GR
3 expression plasmids (0.05 ug/well) in 12 well plates via calcium phosphate
4 precipitation *Chen et al.* (1987) Mol. Cell. Biol. 7, 2745-2752 as described by
5 *Klein et al.* in J. Biol. Chem. 271, 22692, referenced above. The three
6 different RAR-P-GR expression plasmids, pRS-RAR α -P-GR, pcDNA3-
7 RAR β -P-GR and pcDNA3-RAR γ -P-GR, express RAR α , RAR β and RAR γ
8 receptors, respectively, which contain modified DNA binding domains such
9 that their "P-boxes" have been altered to that of the glucocorticoid receptor.
10 These RAR-P-GR receptors bind to DNA as heterodimeric complexes with
11 RXR. Specifically, the RAR-P-GR receptors bind retinoic acid response
12 elements designated R5G, comprised of two RAR half sites (nucleotide
13 sequence 5'-GGTTCA-3') separated by 5 base pairs in which the 3'-half site
14 has been modified to that of a glucocorticoid receptor half site, 5'-AGAACA-
15 3'. To allow for various in transfection efficiency a β -galactosidase
16 expression plasmid (0.01 ug/well) was used as an internal control.
17 Alternatively, the assay was performed in a 96-well microtiter plate format
18 (5000 cells/well) in a manner which was identical to that described above
19 except 1/5 of the amount of the DNA-calcium phosphate precipitant (20 μ l
20 instead of 100 μ l) was applied to each well. Eighteen hours after introduction
21 of the DNA precipitants, cells were rinsed with phosphate buffered saline
22 (PBS) and fed with D-MEM (Gibco-BRL) containing 10% activated charcoal
23 extracted fetal bovine serum (Gemini Bio-Products). Cells were treated for 18
24 hours with the compounds indicated in the figures. After rinsing with PBS
25 cells were lysed with luciferase activity was measured as previously described
26 in *de Wet* (1987) Mol. Cell. Biol. 7, 725-737. Luciferase values represent the
27 mean \pm SEM of triplicate determinations normalized to β -galactosidase
28 activity.

1 Preferred RAR α Selective Agonist Compounds Used in the Invention

2 Presently preferred RAR α specific or selective compounds of the
3 invention are those disclosed in United States Patent No. 5,965,606. The
4 most preferred RAR α specific or selective compounds of the invention are
5 shown in **Formula 1**. These compounds also represent new composition of
6 matter and are considered novel and inventive *per se*. Preferred embodiments
7 of the compounds of the invention within the scope of **Formula 1** are those
8 where the **R** group of **Formula 1** is H or lower alkyl of 1 to 3 carbons, or a
9 pharmaceutically acceptable salt thereof. The most preferred compound of
10 the invention is where the **R** group is H, or a pharmaceutically acceptable salt
11 of said compound. In this connection its noted that a pharmaceutically
12 acceptable salt is any salt which retains the activity of the parent compound
13 and does not impart any deleterious or untoward effect on the subject to which
14 it is administered and in the context in which it is administered.

15 Pharmaceutically acceptable salts may be derived from organic or
16 inorganic bases. The salt may be a mono or polyvalent ion. Of particular
17 interest are the inorganic ions, sodium, potassium, calcium, and magnesium.
18 Organic salts may be made with amines, particularly ammonium salts such as
19 mono-, di- and trialkyl amines or ethanol amines. Salts may also be formed
20 with caffeine, tromethamine and similar molecules.

21 Generally speaking, the compounds of **Formula 1** can be obtained by
22 the synthetic procedures described in United States Patent No. 5,856,490,
23 expressly incorporated by reference. A presently preferred synthetic process
24 for the preparation of the preferred compound of the invention where **R** is H,
25 and of the corresponding ethyl ester is described in detail below.

26 ANTI-PROLIFERATIVE EFFECTS OF THE COMPOUNDS OF THE
27 INVENTION

28 The anti-proliferative effects of the compounds of the invention are
29 demonstrated by assay procedures well accepted in the art. These assays are

1 performed on the preferred compound of the invention, **Compound 2**, also
2 named **AGN 195183** without and in combination with human recombinant α ,
3 β and γ interferon which are anti-tumor agents well known in the art. (The
4 AGN number is a number arbitrarily assigned to compounds in the research
5 laboratories of the assignee of the present invention.) The materials and the
6 assays procedures are described in detail below.

7 The SKBR-3 and T47-D cell cultures in which the assay procedures
8 were performed are also well known and are available from sources well
9 known in the art. Specifically, as is known, T-47D is an estrogen receptor
10 positive (ER⁺) human breast cancer cell line, and SK-BR-3 is an estrogen
11 receptor negative (ER⁻) human breast cancer cell line. The assay procedure
12 which itself is well known in the art, involves determining incorporation of 5-
13 bromo-2'-deoxyuridine (BrdU) into the cells. As is known, incorporation of
14 less BrdU represents less cell proliferation (inhibition of cell proliferation),
15 and this assay is accepted in the art as a measure of anti-proliferative or anti-
16 tumor activity of the assayed agent or agents.

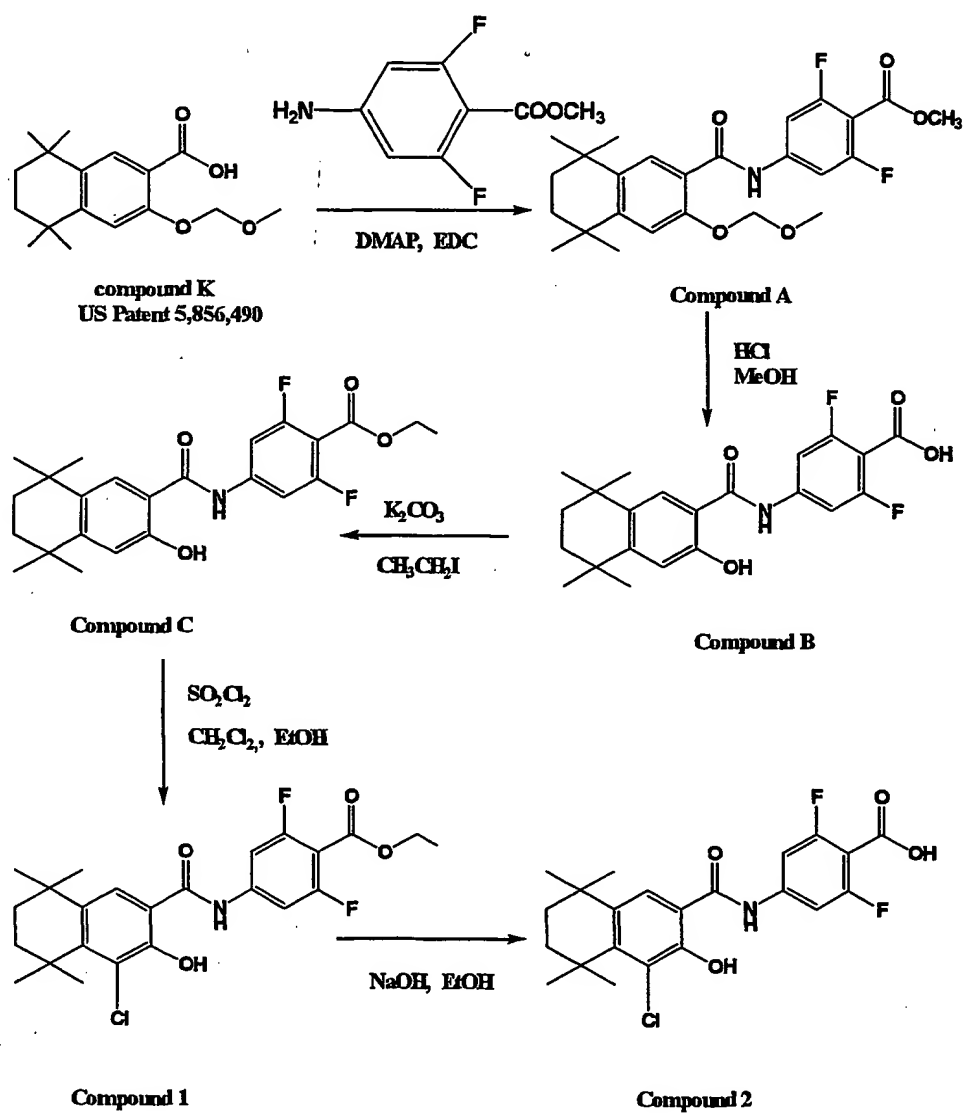
17 When a combination of two or more anti-proliferative or potentially
18 anti-proliferative agents is assayed, the results may indicate less inhibition of
19 proliferation than what we would be expected if the effects of the individual
20 agents were additive, or the effects may represent the mathematical product of
21 the expected effects of the two agents (additive inhibition). Alternatively, the
22 inhibition actually observed experimentally may be greater than what would
23 be expected as a simple product of the effects of the two agents. Such
24 synergistic anti-tumor or antiproliferative effect is highly desirable, and as is
25 described below was observed in several assays when **Compound 2** of the
26 invention was used in combination with human recombinant interferon. This
27 synergistic effect of the compounds with interferon in the treatment of tumors,
28 and especially of breast cancer, is not expected based on the prior art and is
29 unobvious and surprising. The materials and procedures of the assays as well

1 as the mathematical criteria for determining synergistic effects are described
2 below.

3 Materials, Assay Methods and Criteria for Determining Synergism 4 Reagents

5 The human recombinant interferon-alpha (IFN- α) and human
6 recombinant interferon-beta (IFN- β) were purchased from Sigma Chemicals
7 Co. (St Louis, MO). Human recombinant interferon-gamma (IFN- γ) was
8 purchased from Roche Diagnostics (Indianapolis, IN). The stock solutions
9 were stored at -70, 4, and -20 °C for IFN- α , IFN- β and IFN- γ , respectively.
10 IFN working solutions were prepared before use by dilutions in the culture
11 medium. 5 mM stock solution for **Compound 2** (AGN195183) was prepared
12 in DMSO, which was subsequently diluted in culture medium to the indicated
13 final concentration.

14 Synthesis of Preferred Compounds (Reaction Scheme 1)



1 Methyl 2,6-difluoro-4-[(3-methoxymethoxy-5,5,8,8,-tetramethyl-5,6,7,8-
2 tetrahydro-naphthalene-2-carbonyl)-amino]-benzoate (Compound A)

3 To a solution of 3-methoxymethoxy-5,5,8,8,-tetramethyl-5,6,7,8-
4 tetrahydro-naphthalene-2-carboxylic acid (Compound K, as described in
5 United States Patent No. 5,856,490, 112mg, 0.38 mmol) in 6 ml of anhydrous
6 methylene chloride was added 4-(dimethylamino)pyridine (DMAP, 100mg,
7 0.46mmol), methyl 2,6-difluoro-4-aminobenzoate (Compound H1, as
8 described in United States Patent No. 5,856,490, 77mg, 0.38mmol) and 1-
9 (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 110mg,
10 0.57mmol). The reaction mixture was stirred at room temperature for
11 overnight then concentrated to dryness. The residue was purified by column
12 chromatography with ethyl acetate : hexane (1:9) to yield the title compound
13 as a clear oil.

14 ¹H NMR CDCl₃ δ 8.18 (s, 1H), 7.38 (s, 1H), 7.35 (s, 1H), 7.10 (s, 1H), 5.39 (s,
15 2H), 3.94 (s, 3H), 3.59 (s, 3H), 1.70 (s, 4H), 1.31 (s, 3H), 1.30 (s, 3H).

16 2,6-difluoro-4-[(3-hydroxy-5,5,8,8,-tetramethyl-5,6,7,8-tetrahydro-
17 naphthalene-2-carbonyl)-amino]-benzoic acid (Compound B)

18 A solution of methyl 2,6-difluoro-4-[(3-methoxymethoxy-5,5,8,8,-
19 tetramethyl-5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-amino]-benzoate
20 (Compound A, 113mg, 0.26mmol) in 6 ml of methanol and 3 drops of conc.
21 HCl was stirred at room temperature for overnight and then concentrated to
22 dryness. The solid was recrystallized from ethyl ether : hexane to give the title
23 compound as a white solid.

24 ¹H NMR acetone-d₆ δ 10.2 (bs, 1H), 7.94 (s, 1H), 7.56 (s, 1H), 7.53 (s, 1H),
25 6.94 (s, 1H), 1.69 (s, 4H), 1.27 (s, 6H).

26 Ethyl 2,6-difluoro-4-[(3-hydroxy-5,5,8,8,-tetramethyl-5,6,7,8-tetrahydro-
27 naphthalene-2-carbonyl)-amino]-benzoate (Compound C)

28 To a solution of 2,6-difluoro-4-[(3-hydroxy-5,5,8,8,-tetramethyl-

1 5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-amino]-benzoic acid (**Compound**
2 **B**, 56mg, 0.13mmol) in 4 ml of acetone was added potassium carbonate (
3 36mg, 0.26mmol) and iodoethane (0.012ml, 0.14mmol). The reaction mixture
4 was stirred at room temperature for 4 hours then concentrated and purified by
5 column chromatography with ethyl acetate: hexane (1:9) to yield the title
6 compound as a white solid.

7 ¹H NMR CDCl₃ δ 8.00 (s, 1H), 7.38 (s, 1H), 7.35 (s, 1H), 6.95 (s, 1H), 4.40 (q,
8 J=7.1 Hz, 2H), 1.70 (s, 4H), 1.41 (t, J=7.2 Hz, 3H), 1.31 (s, 3H), 1.29 (s, 3H).
9 Ethyl 2,6-difluoro-4-[(3-hydroxy-4-chloro-5,5,8,8-tetramethyl-5,6,7,8-
10 tetrahydro-naphthalene-2-carbonyl)-amino]-benzoate (Compound 1)

11 To a solution ethyl 2,6-difluoro-4-[(3-hydroxy-5,5,8,8-tetramethyl-
12 5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-amino]-benzoate (**Compound C**,
13 227 mg, 0.52 mmol) in 10 ml of anhydrous dichloromethane under nitrogen at
14 25°C was added sulfonyl chloride (0.0413 ml, 0.57 mmol) and anhydrous
15 ethyl ether (0.054 ml, 0.52 mmol). Reaction was instantaneous at 25°C as
16 monitored by ¹H NMR. The reaction mixture was quenched with saturated
17 NaHCO₃ then extracted with ethyl acetate. The organic layer was washed with
18 water, brine and dried over Na₂SO₄. The title compound was obtained as a
19 white solid after column chromatography with ethyl acetate : hexane (1:9).
20 ¹H NMR CDCl₃ δ 9.33 (b, 1H), 8.56 (b, 1H), 7.90 (s, 1H), 7.36 (d, J=9.83 Hz,
21 2H), 4.39 (q, J=7.1 Hz, 2H), 1.75 (m, 2H), 1.65 (m, 2H), 1.53 (s, 6H), 1.39 (t,
22 J=7.2 Hz, 3H), 1.32 (s, 6H).

23 2,6-Difluoro-4-[(3-hydroxy-4-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-
24 naphthalene-2-carbonyl)-amino]-benzoic acid (Compound 2)

25 To a solution of ethyl 4-[(4-chloro-3-hydroxy-5,5,8,8-tetramethyl-
26 5,6,7,8-tetrahydro-naphthalene-2-carbonyl)-amino]-2,6-difluoro-benzoate
27 (**Compound 1**, 150 mg, 0.32 mmol) in 6 ml of EtOH was added 2ml of 2M
28 NaOH(aq). The reaction was stirred at room temperature for 12 hours then

1 acidified with 10% HCl to PH=5. The excess alcohol was removed by
2 evaporation in a rotary apparatus and the aqueous layer was extracted with
3 ethyl acetate (3x10ml). The combined organic layers were washed with water,
4 brine, and dried over Na₂SO₄. After evaporation of the solvent, the title
5 compound was obtained in a crude form and was recrystallized in ethyl acetate
6 / hexane to afford the pure title compound (AGN 195183) as a light yellow
7 solid.

8 ¹H NMR Acetone-d₆ δ 7.97(s, 1H), 7.53(d, J=10.2 Hz, 2H), 1.75 (m, 2H),
9 1.65 (m, 2H), 1.54 (s, 6H), 1.31 (s, 6H).

10 Culture of Breast Cancer Cell Lines

11 The estrogen receptor-positive (ER⁺) cell line T-47D and the ER⁻ cell
12 line SK-BR-3 were cultured in Dulbecco's modification of Eagle's medium
13 (DMEM Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine
14 serum (HyClone, Logan, UT), 2 mM L-glutamine and 1% antibiotics-
15 antimycotics (Gibco BRL). Cell lines were obtained from the American Type
16 Culture Collection (ATCC, Rockville, MD, HTB-133 and HTB-30 for T47-D
17 and SKBR-3, respectively). Cells were cultured at 37 °C in a humidified
18 atmosphere containing 5% CO₂.

19 Cell Proliferation Assay

20 Proliferation of cancer cell lines was determined using a commercial
21 cell proliferation kit (Roche Diagnostics), essentially following the
22 instructions of the manufacturer. Cells were seeded into 96-well tissue culture
23 plates (Corning Incorporated, Corning, NY) at a concentration of 3000
24 cells/well. After 24 hours, cells were treated with **Compound 2** (AGN195183)
25 and/or interferons (IFNs) or solvent alone. The appropriate concentrations of
26 **Compound 2** (AGN195183) used in this study were between 10⁻¹¹M and
27 10⁻⁶M; IFNs concentrations were between 25 and 1000 Unit/ml. Culture media
28 were changed every 72 hours. After 7days, 10 µl of 5-bromo-2'-deoxyuridine

1 (BrdU) was added to each well. Incubation with BrdU was stopped 24 hours
2 later by adding 100 μ l of anti-BrdU antibody to each well. The amount of
3 BrdU incorporated into the DNA of proliferating cells was assessed by
4 measuring absorbance at 450 nm. Each experiment was performed in
5 triplicate.

6 Criteria for Synergism

7 The growth inhibition observed in the cell cultures as a result of
8 treatment with a combination of **Compound 2** (AGN195183) of the invention
9 and the interferons (IFNs) was analyzed for synergistic and additive effects.
10 Synergistic effects were determined by calculating the ratio between the
11 percentage of cell growth expected assuming an additive interaction and the
12 actual cell growth observed when combining both agents (values > 1 indicates
13 synergistic actions). Statistical significance of synergistic effects were
14 determined using two-sided student's t-test.

15 Synergism was defined as: $\%A \times \%B > \%AB$

16 Additivity was defined as: $\%A \times \%B = \%AB$

17 where A and B are the effects of each individual agent and AB is the
18 effect of the combination, in accordance with the teaching of *Aapro et al.*,
19 *Cancer Chemother. Pharmacol.*, 10: 161-166, 1983, and *Marth et al.*, *J. Natl.*
20 *Cancer Inst.*, 77:1197-1202, 1986), both of which are expressly incorporated
21 herein by reference.

22 Anti-Proliferative Effects Determined by the Assays

23 Referring now to the graphs of **Figures 1** through **12**, each of these
24 represents the results obtained in the above described assays where SKBR-3
25 and T47-D cells, respectively, were treated with a combination of
26 **Compound 2** of the invention and human recombinant interferon (IFN) α , β ,
27 and γ , respectively. The graph of **Figure 13** illustrates the results of
28 treatment of these two cell cultures only with **Compound 2** of the invention,

1 without the use of any other anti-tumor agent. In each of these graphs the
2 incorporation of 5-bromo-2'-deoxyuridine (BrdU) is plotted on the Y
3 (vertical) axis and varying concentration of **Compound 2** of the invention or
4 varying concentration of IFN α , IFN β or of IFN γ , respectively is plotted on
5 the X (horizontal) axis. The concentration of the interferons is expressed in
6 international units, as is accepted in the art, whereas the molar concentration
7 of **Compound 2** is plotted on a logarithmic scale. Each graph, except for the
8 graph of **Figure 13**, includes a curve indicating results with one agent only,
9 actual experimental results with the combination of the two agents
10 (**Compound 2** and the respective interferon), and a theoretical curve which is
11 calculated in the manner described above, assuming for the calculation that
12 the effects of the two agents would be simply additive. The incorporation of
13 BrdU is plotted on a percentage basis relative to the situation when the agent
14 of varying concentration in the respective graph was not used (0 concentration
15 represents 100 % incorporation).

16 Referring now specifically to the graph of **Figure 1**, in the assay in
17 SKBR-3 cells depicted in that graph the concentration of **Compound 2** was
18 10 nanomolar (nM), and the concentration of the IFN α was varied. It can be
19 seen on the graph that the experimentally or actually observed inhibition of
20 cell proliferation was significantly greater (less BrdU incorporation) than with
21 IFN α alone, and significantly greater than the theoretically additive curve,
22 thus showing a synergistic effect of **Compound 2** and IFN α . The graphs of
23 **Figures 2 and 3**, similarly depict the results of assays in SKBR-3 cells where
24 the concentration of **Compound 2** was kept constant at 10 nM and the
25 concentration of IFN β or IFN γ , respectively, was varied. The graphs of
26 **Figures 2 and 3** also show significant synergistic effect of the combination
27 treatment.

28 The graphs of **Figures 4, 5 and 6** disclose the results of assays in
29 SKBR-3 cells where the concentration of IFN α , IFN β and of IFN γ ,

1 respectively, was kept constant at 100 international units per ml (U/ml), and
2 the concentration of **Compound 2** of the invention was varied. These graphs
3 also show significant synergistic effect, representing that the combination of
4 the interferon and of **Compound 2** inhibits cell proliferation significantly
5 more than what would be expected based on the individual effects of these
6 two agents.

7 The graphs of **Figures 7, 8 and 9** disclose results of assays in T47-D
8 cells as a result of treatment with a combination of a constant concentration
9 (100 nM) of **Compound 2**, and varying concentration of IFN α , IFN β and of
10 IFN γ , respectively. The graph of **Figure 7** reveals that inhibition by the
11 combination is additive in this cell line when IFN α is used. However, when
12 IFN β and IFN γ were used, the observed inhibition was significantly
13 synergistic.

14 **Figures 10, 11 and 12** disclose the results of assays in T47-D cells
15 where the concentration of IFN α , IFN β and of IFN γ , respectively, was kept
16 constant at 100 international units per ml (U/ml), and the concentration of
17 **Compound 2** of the invention was varied. When IFN α was used in the
18 combination (**Figure 10**) the combination was not very effective and merely
19 additive, but when IFN β and IFN γ were used, again synergistic inhibition
20 was observed, in the cotreatment with IFN γ only at higher concentrations of
21 **Compound 2**.

22 The foregoing results and particularly the synergism in the anti-
23 proliferative effects on these two cancer cell lines of the compounds of the
24 invention and of human recombinant interferon is unexpected, surprising, and
25 an indication that RAR α specific or RAR α selective compounds, and
26 particularly the preferred compounds of the invention are useful for the
27 treatment of diseases involving malignant cell-proliferation, such as
28 carcinomas and particularly carcinoma of the breast. In fact, the foregoing
29 assays indicated that RAR α specific or RAR α selective compounds, and

1 particularly the preferred compounds of the invention are useful in
2 combination therapy with interferon in breast cancer cell lines which are
3 estrogen receptor positive (T-47D) and also in human breast cancer cell lines
4 which are estrogen receptor negative (SK-BR-3).

5 Methods of Treatment, Modes of Administration

6 The $\text{RAR}\alpha$ specific or $\text{RAR}\alpha$ selective compounds, and particularly
7 the preferred compounds may be administered, in accordance with the present
8 invention, systemically or topically, depending on such considerations as the
9 condition to be treated, need for site-specific treatment, quantity of drug to be
10 administered, and numerous other considerations. For the treatment of breast
11 cancer and many other forms of malignant tumors the compounds are more
12 likely to be administered systemically, in a pharmaceutical composition
13 containing such excipients or inert components which are well known in the
14 art pertaining to chemotherapy of tumors. More specifically, if the compound
15 is to be administered systemically, it may be confected as a powder, pill, tablet
16 or the like or as a syrup or elixir suitable for oral administration. For
17 intravenous or intraperitoneal administration, the compound will be prepared
18 as a solution or suspension capable of being administered by injection. In
19 certain cases, it may be useful to formulate these compounds by injection. In
20 certain other cases, it may be useful to formulate these compounds in
21 suppository form or as extended release formulation for deposit under the skin
22 or intramuscular injection.

23 The $\text{RAR}\alpha$ specific or $\text{RAR}\alpha$ selective compounds, and
24 particularly the preferred compound of the invention will be administered as a
25 chemotherapeutic agent for treatment of tumors in a useful therapeutic dose
26 which will vary from condition to condition and in certain instances may vary
27 with the severity of the condition being treated and the patient's susceptibility
28 to treatment. Accordingly, no single dose will be uniformly useful, but will
29 require modification depending on the particularities of the tumor or

1 malignancy being treated. Such doses can be arrived at through routine
2 experimentation. For the treatment of solid tumors, particularly breast cancer
3 it is anticipated that the compound will be administered for approximately 1 to
4 8 weeks to a patient in need thereof, in a dose that is effective to halt, slow the
5 growth or dissipate the tumor. Preferably the compound is to be administered
6 orally, in a daily dose which preferably will be in the range of a approximately
7 50 mg per day to 500 mg per day. Most preferably the compound used in the
8 treatment will be **Compound 2** of the invention.

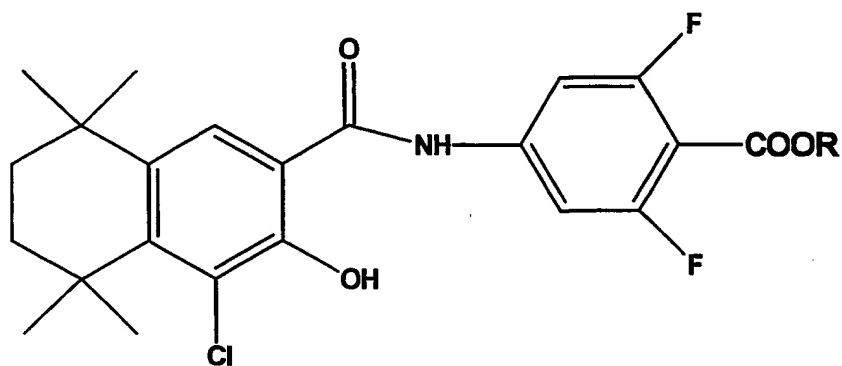
9 Preferably the RAR α specific or RAR α selective compounds, and
10 particularly the preferred compounds of the invention, and most preferably
11 **Compound 2**, will be administered, in accordance with the invention, in
12 combination with other chemotherapeutic agents, such as interferons,
13 preferably human recombinant interferon, or other known chemotherapeutic
14 agents of tumors. Other chemotherapeutic agents with which the compounds
15 are likely to be used in combination therapy are: tamixofen and taxol. With
16 the use of interferons and with certain other chemotherapeutic agents as well,
17 a synergistic anti-tumor effect is likely to occur, as is demonstrated by the
18 above described cell culture assay procedures. Again, when the compounds
19 are used in a combination therapy the useful therapeutic dose will vary from
20 condition to condition and in certain instances may vary with the severity of
21 the condition being treated and the patient's susceptibility to treatment.
22 Accordingly, the required dose will be arrived at through routine
23 experimentation, which is customary in the science of chemotherapy of
24 tumors.

25 Generally speaking it is contemplated that in combination therapy and
26 for the treatment of solid tumors such as breast cancer, the daily dose of the
27 compound will be in the range of a approximately 50 mg per day to 500 mg
28 per day. The daily dose of the other chemotherapeutic agent or agents given
29 in combination with the compound of the invention will depend on the nature

1 of the chemotherapeutic agent or agents, and can be arrived by routine
2 experimentation normally practiced in the art. When interferon is used for the
3 treatment of solid tumors, such as for example breast cancer, in combination
4 with RAR α specific or RAR α selective compounds, and particularly with the
5 preferred the compounds of the invention, then the daily dose of the
6 interferon is likely to be in the range of approximately 1 to 9 million
7 international units per day.

WHAT IS CLAIMED IS:

1. A compound of the formula

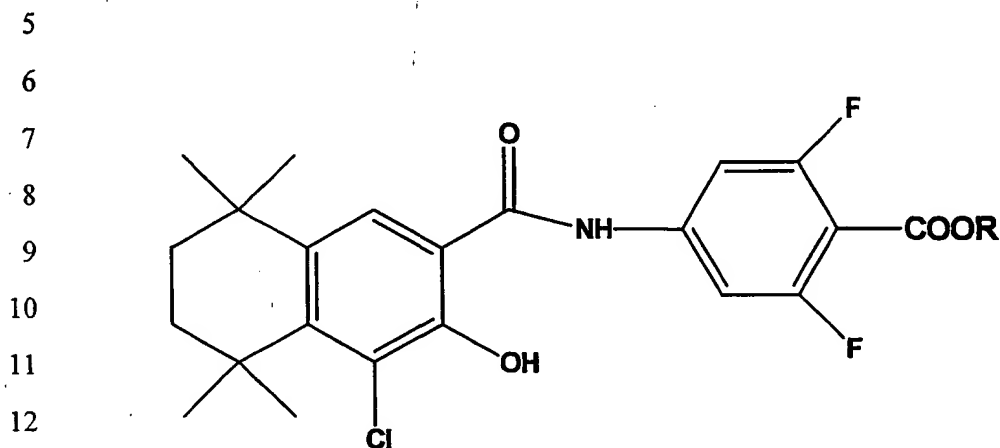


wherein R is a H, lower alkyl of 1 to 6 carbons, or a pharmaceutically acceptable salt of said compound.

2. A compound in accordance with Claim 1 wherein R is lower alkyl of 1 to 3 carbons.

3. A compound in accordance with Claim 1 wherein R is H, or a pharmaceutically acceptable salt of said compound.

- 1 4. A pharmaceutical composition for the treatment of a malignant
2 disease or condition in a mammal, the composition comprising a
3 pharmaceutically acceptable excipient and a therapeutically effective dose of a
4 compound of the formula



- 14 wherein R is a H, lower alkyl of 1 to 6 carbons, or a pharmaceutically
15 acceptable salt of said compound.

- 16 5. A pharmaceutical composition in accordance with Claim 4 wherein
17 in the formula of the compound R is lower alkyl of 1 to 3 carbons.

- 18 6. A pharmaceutical composition in accordance with Claim 4 wherein
19 in the formula of the compound R is H, or a pharmaceutically acceptable
20 salt of said compound.

- 21 7. A pharmaceutical composition in accordance with Claim 4 further
22 comprising a chemotherapeutic agent effective for the treatment of the
23 malignant disease or condition of the mammal.

- 24 8. A pharmaceutical composition in accordance with Claim 5 further
25 comprising a chemotherapeutic agent effective for the treatment of the
26 malignant disease or condition of the mammal.

- 27 9. A pharmaceutical composition in accordance with Claim 6 further
28 comprising a chemotherapeutic agent effective for the treatment of the
29 malignant disease or condition of the mammal.

1 **10.** A pharmaceutical composition in accordance with Claim 7
2 wherein the chemotherapeutic agent effective for the treatment of the
3 malignant disease or condition of the mammal is interferon.

4 **11.** A pharmaceutical composition in accordance with Claim 8
5 wherein the chemotherapeutic agent effective for the treatment of the
6 malignant disease or condition of the mammal is interferon.

7 **12.** A pharmaceutical composition in accordance with Claim 9
8 wherein the chemotherapeutic agent effective for the treatment of the
9 malignant disease or condition of the mammal is interferon.

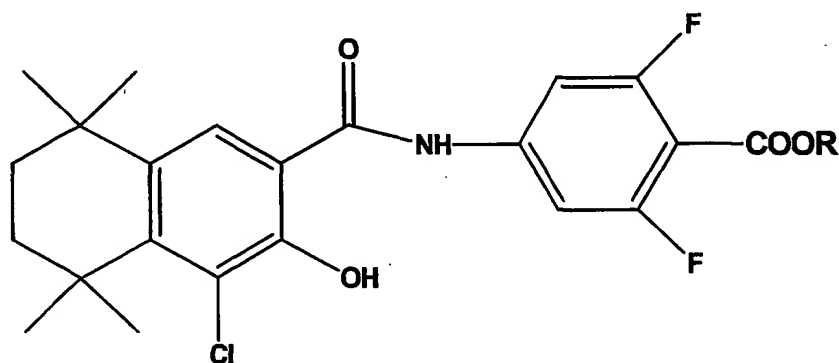
10 **13.** A pharmaceutical composition in accordance with Claim 4
11 comprising a daily dose of approximately 50 mg to 500 mg of the compound.

12 **14.** A pharmaceutical composition in accordance with Claim 4
13 adapted for the treatment of breast cancer.

14 **15.** A pharmaceutical composition in accordance with Claim 9
15 wherein the chemotherapeutic agent effective for the treatment of the
16 malignant disease or condition of the mammal is human recombinant
17 interferon α , human recombinant interferon β , or human recombinant
18 interferon γ .

19 **16.** A pharmaceutical composition in accordance with Claim 15
20 adapted for the treatment of breast cancer.

1 17. A method of combination therapy for treating a malignant disease
2 or condition in a mammal in need of such treatment, the method comprising
3 the steps of administering to the mammal
4 a pharmaceutical composition comprising a pharmaceutically
5 acceptable excipient and a therapeutically effective dose of a compound of the
6 formula



17 wherein **R** is a H, lower alkyl of 1 to 6 carbons, or a pharmaceutically
18 acceptable salt of said compound, and
19 co-administering to the mammal another chemotherapeutic agent
20 effective for the treatment of the malignant disease or condition of the
21 mammal.

22 18. A method in accordance with Claim 17 wherein in the formula of
23 the compound **R** is lower alkyl of 1 to 3 carbons.

24 19. A method in accordance with Claim 17 wherein in the formula of
25 the compound **R** is H, or a pharmaceutically acceptable salt of said compound.

26 20. A method in accordance with Claim 17 wherein a daily dose of
27 approximately 50 mg to 500 mg of the compound is administered to the
28 mammal.

29 21. A method in accordance with Claim 17 wherein the malignant

1 disease or condition of the mammal is breast cancer.

2 **22.** A method in accordance with Claim 17 wherein the other
3 chemotherapeutic agent is interferon.

4 **23.** A method in accordance with Claim 21 wherein the other
5 chemotherapeutic agent is interferon.

6 **24.** A method of combination therapy for treating a malignant disease
7 or condition in a mammal in need of such treatment, the method comprising
8 the steps of administering to the mammal
9 a pharmaceutical composition comprising a pharmaceutically
10 acceptable excipient and a therapeutically effective dose of a compound that is
11 specific or selective agonist of RAR α receptors in preference over RAR β and
12 RAR γ receptors, and
13 co-administering to the mammal another chemotherapeutic agent
14 effective for the treatment of the malignant disease or condition of the
15 mammal.

16 **25.** A method in accordance with Claim 24 wherein a daily dose of
17 approximately 50 mg to 500 mg of the RAR α specific or selective compound
18 is administered to the mammal.

19 **26.** A method in accordance with Claim 24 wherein the malignant
20 disease or condition of the mammal is breast cancer.

21 **27.** A method in accordance with Claim 24 wherein the other
22 chemotherapeutic agent is interferon.

23 **28.** A method in accordance with Claim 24 wherein the malignant
24 disease or condition of the mammal is breast cancer, the other
25 chemotherapeutic agent is interferon and a daily dose of approximately 50 mg
26 to 500 mg of the RAR α specific or selective compound is administered to the
27 mammal.

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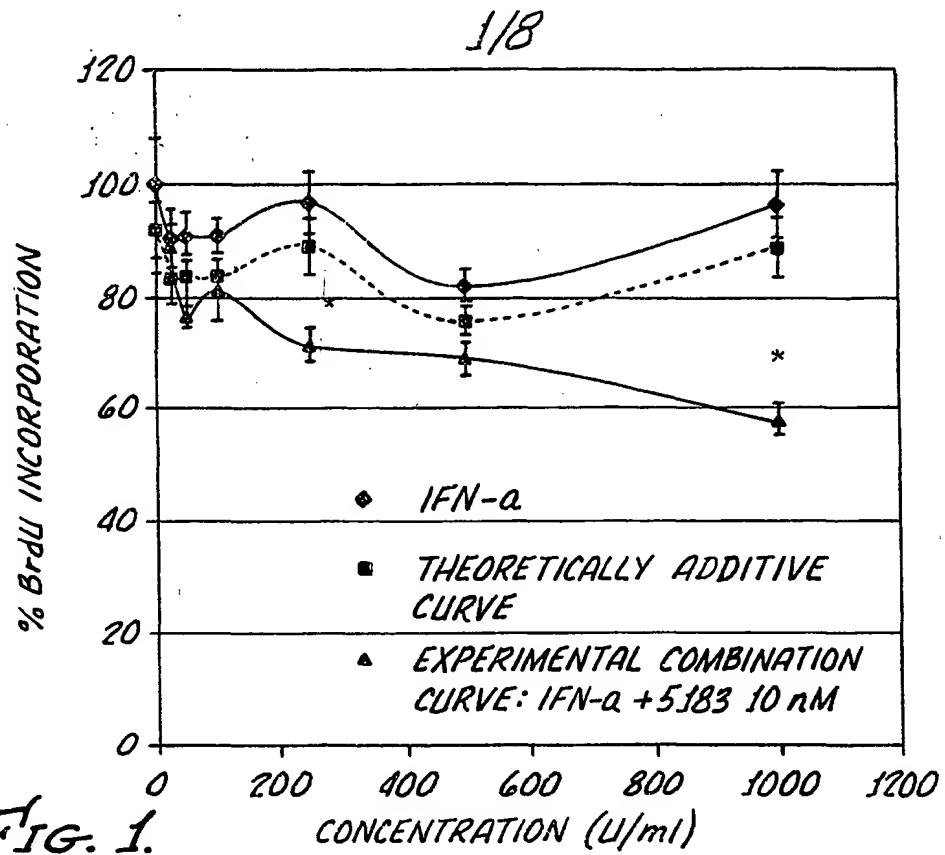


FIG. 1.

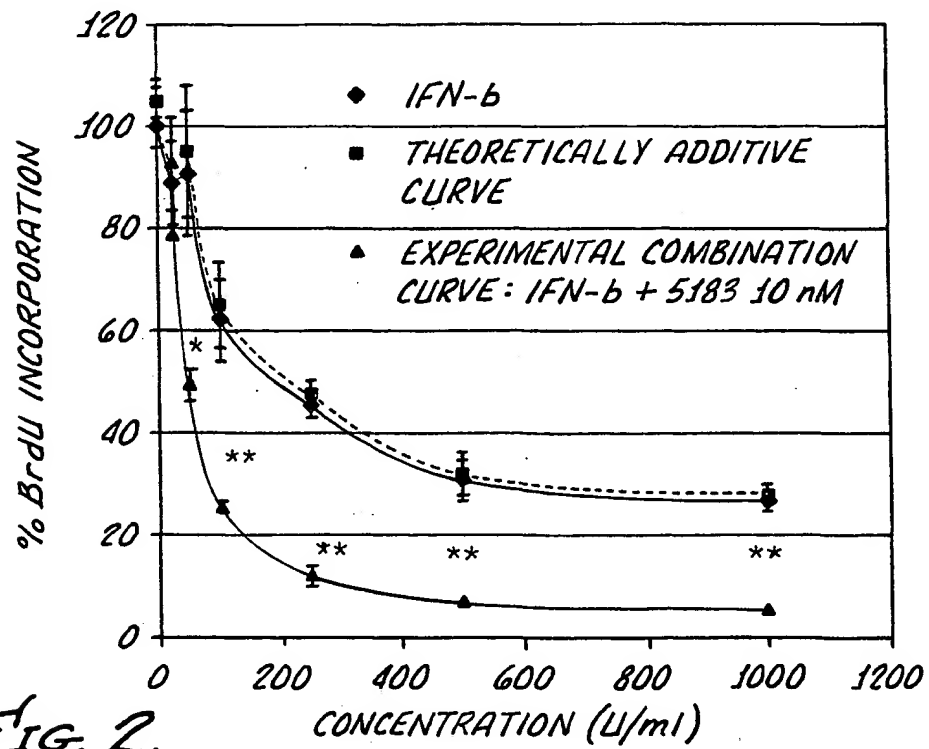


FIG. 2.

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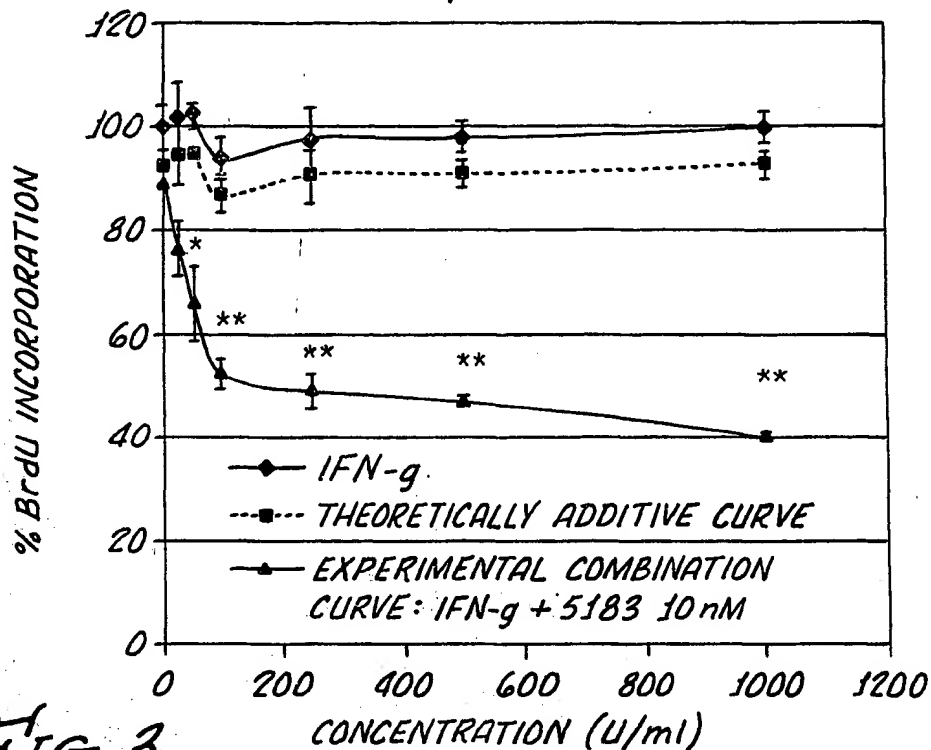
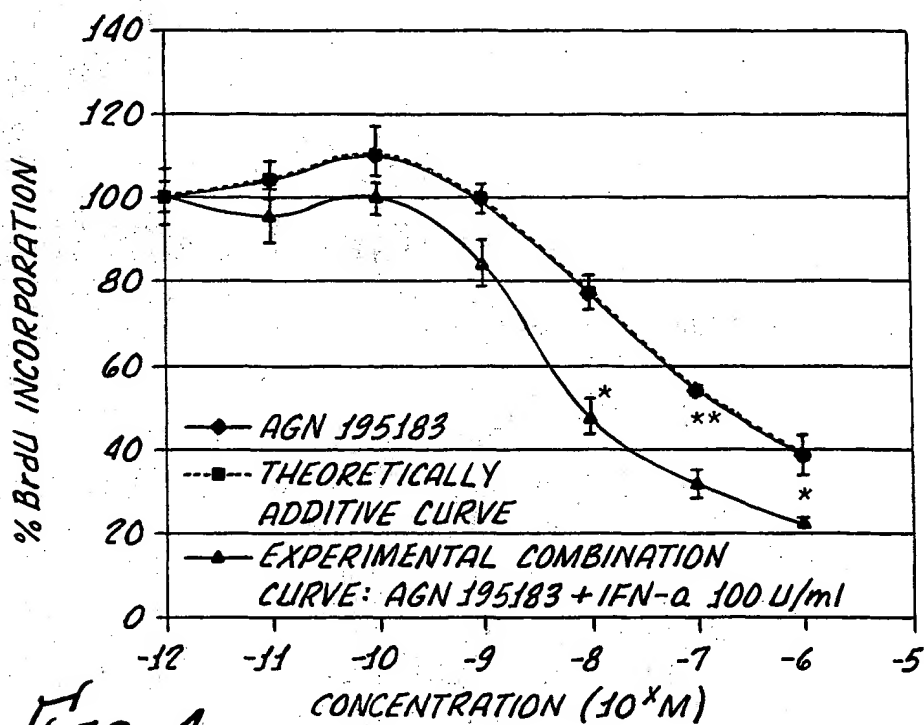


FIG. 4.



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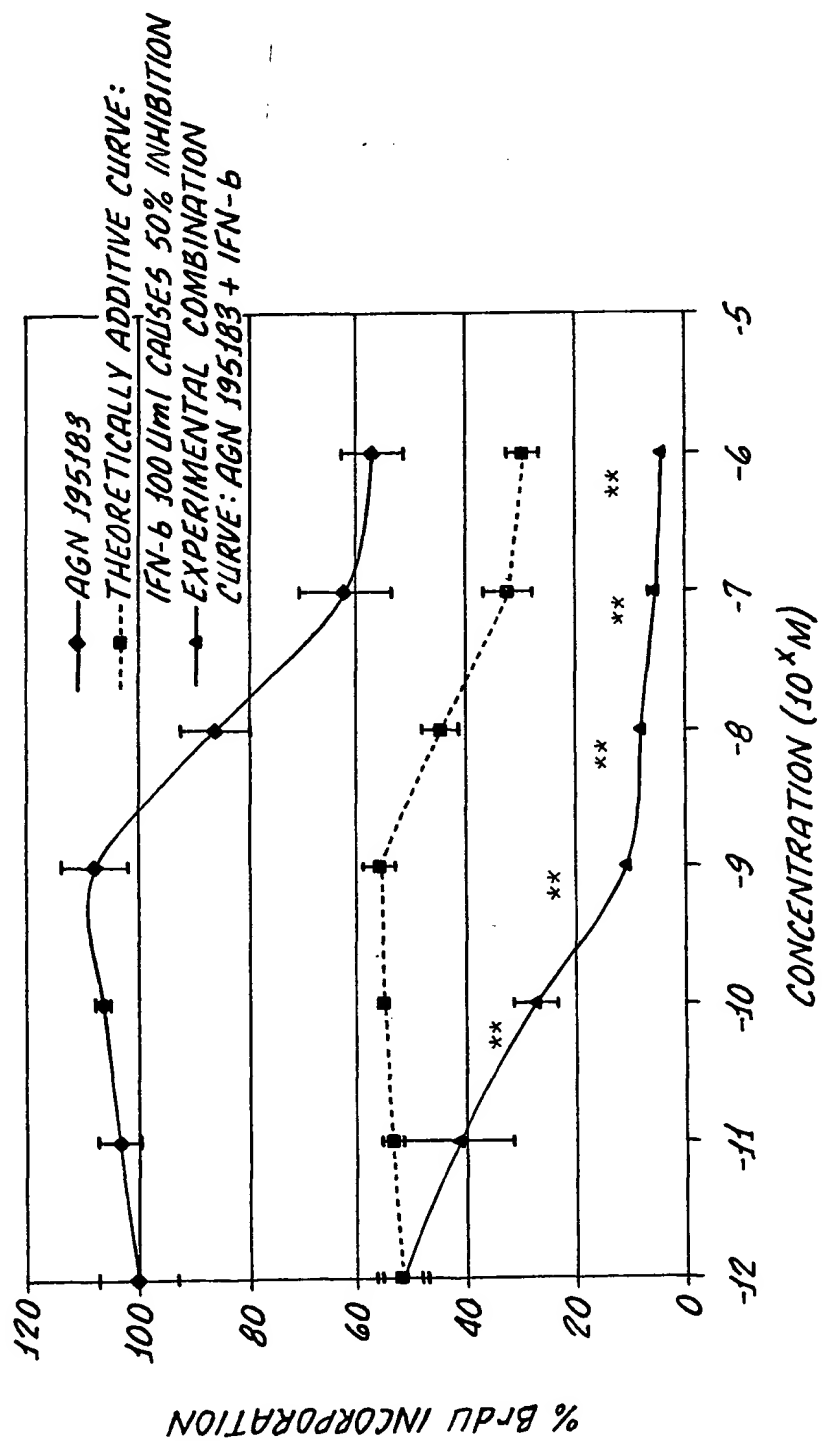


FIG. 5.

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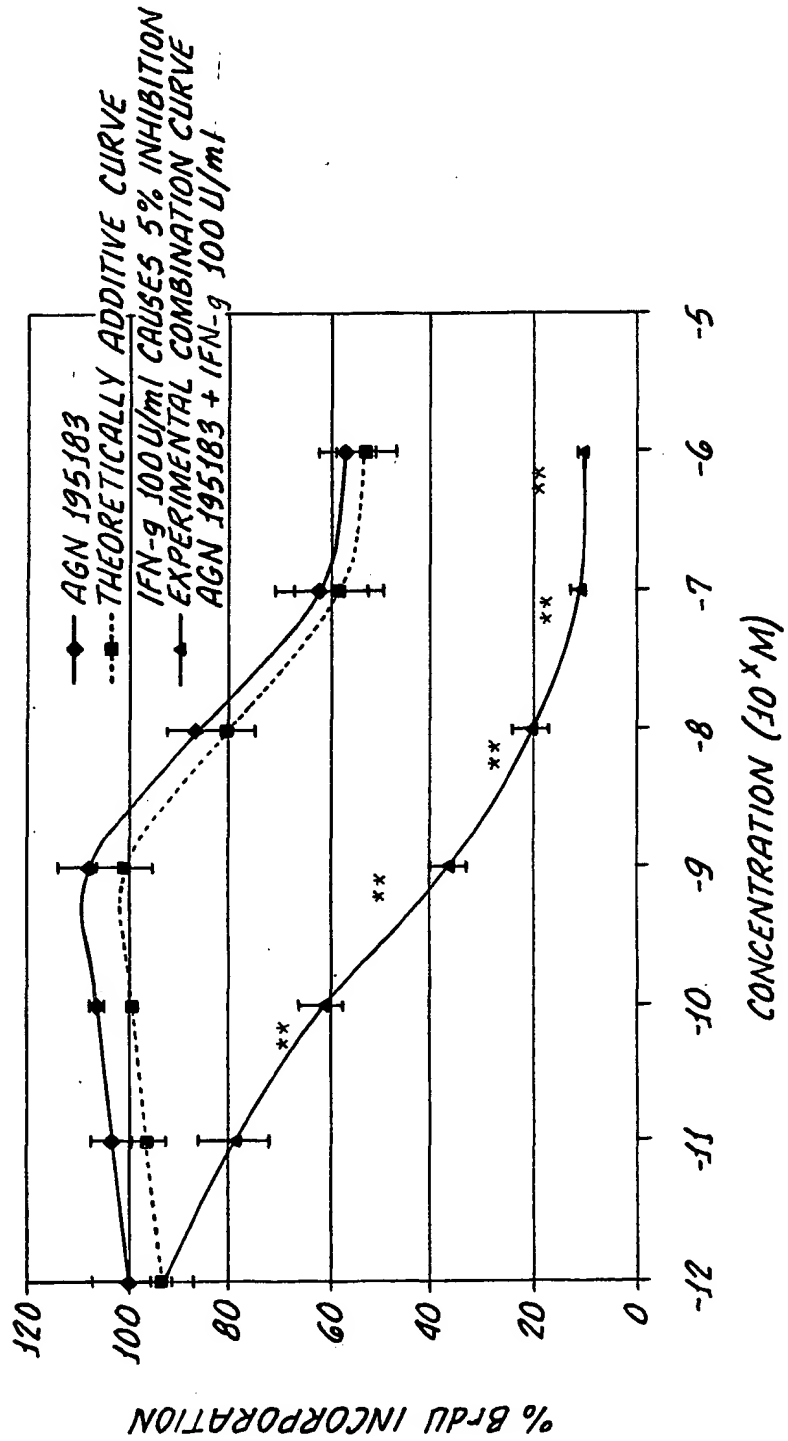


FIG. 6.

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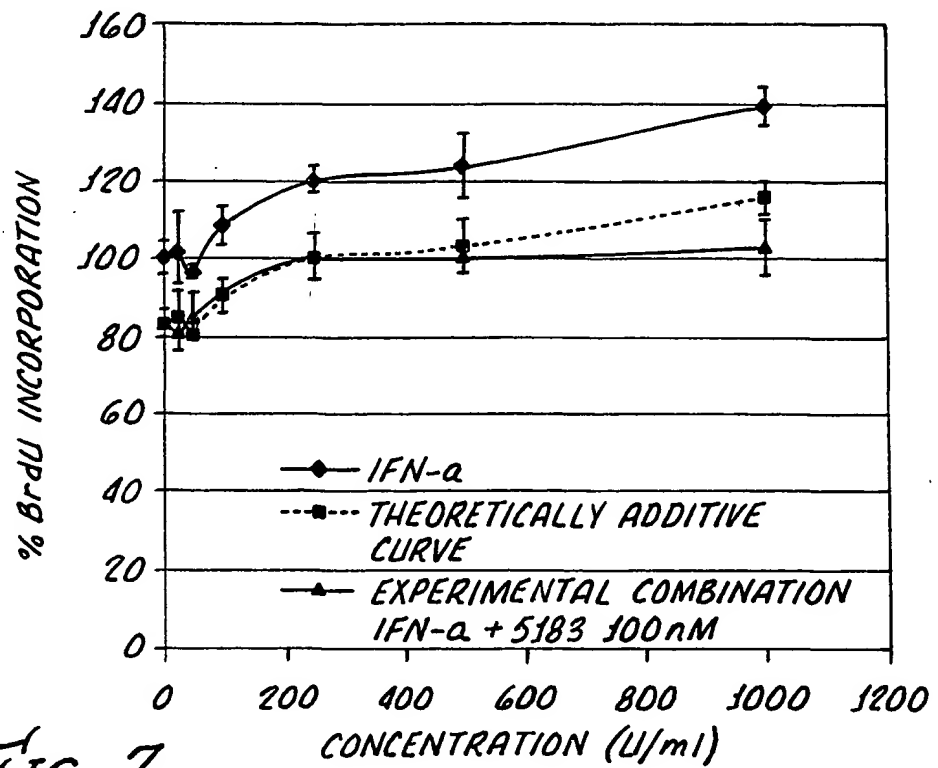


FIG. 7.

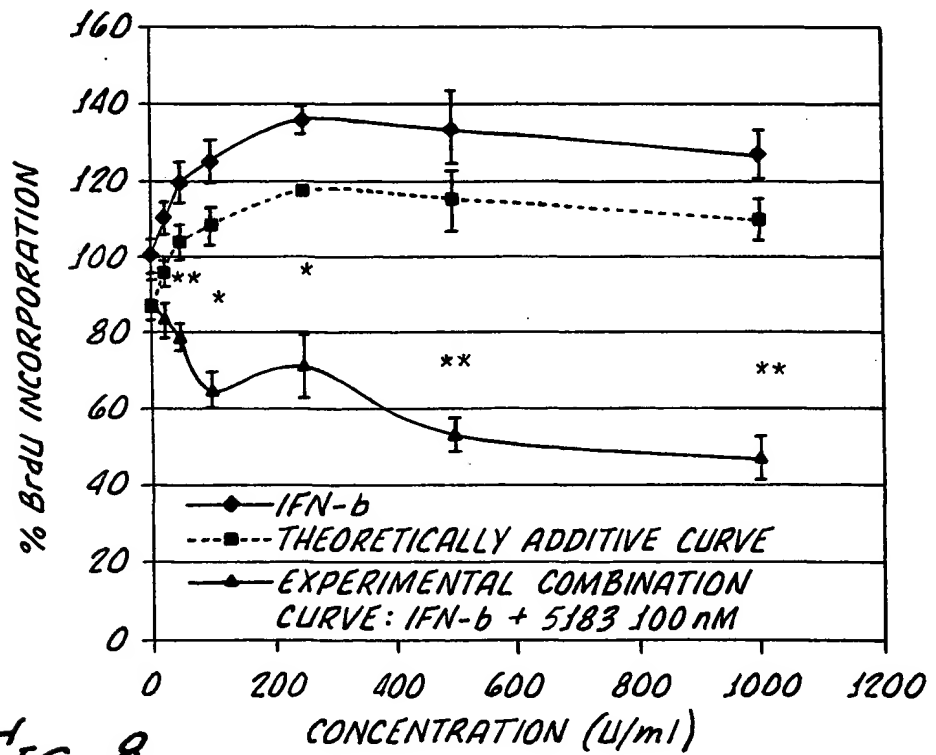


FIG. 8.

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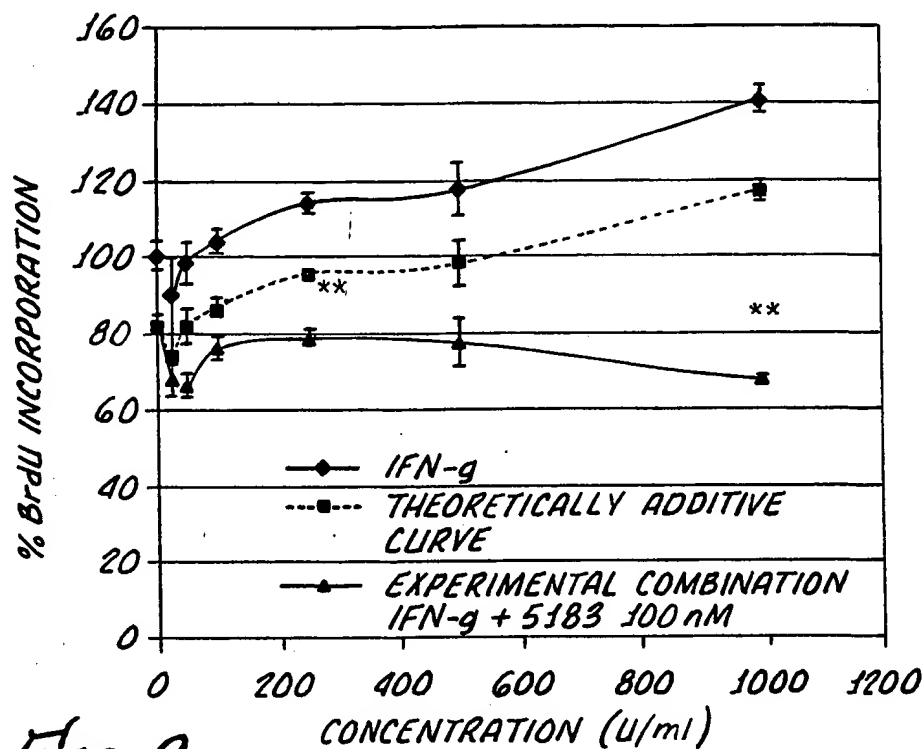


FIG. 9.

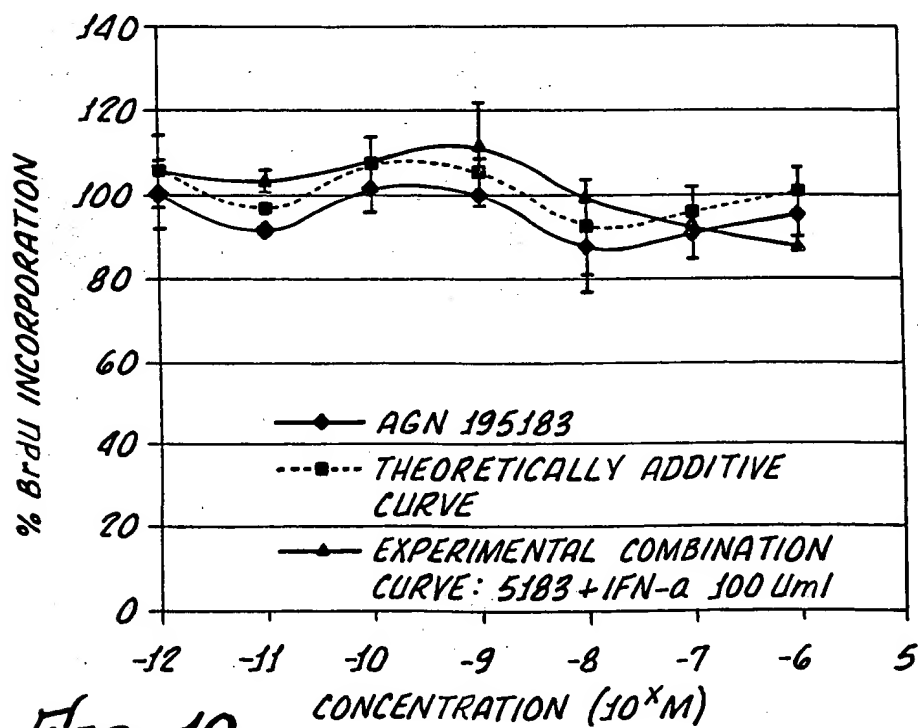


FIG. 10.

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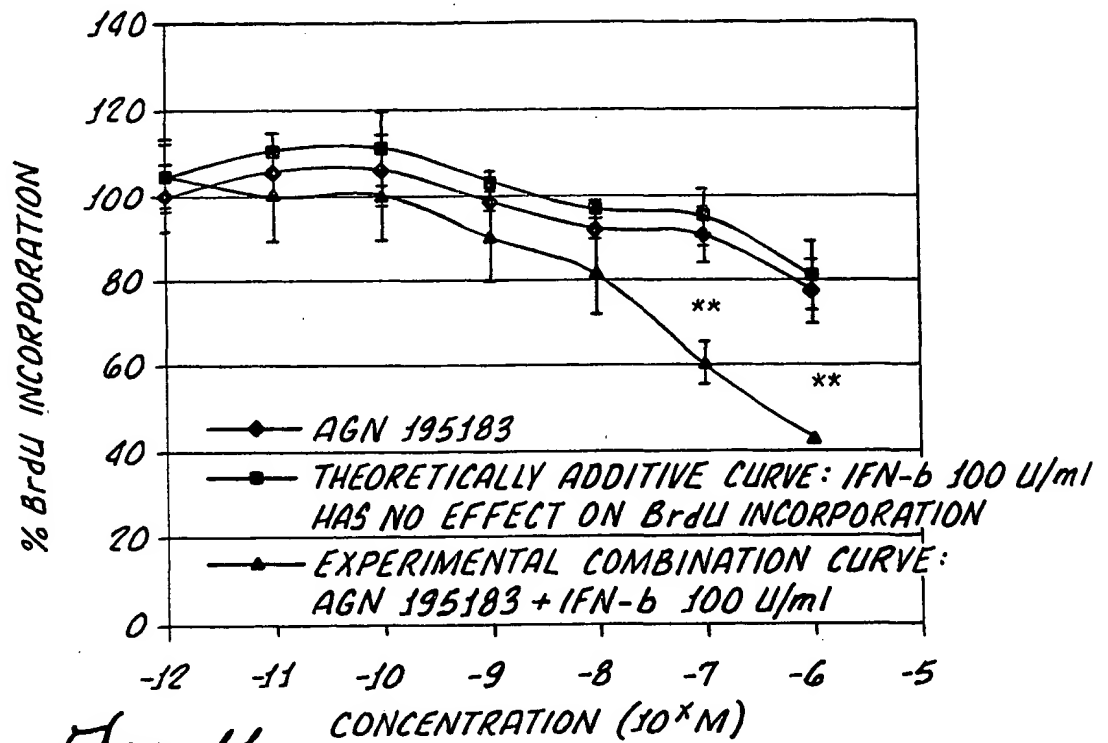


FIG. 11.

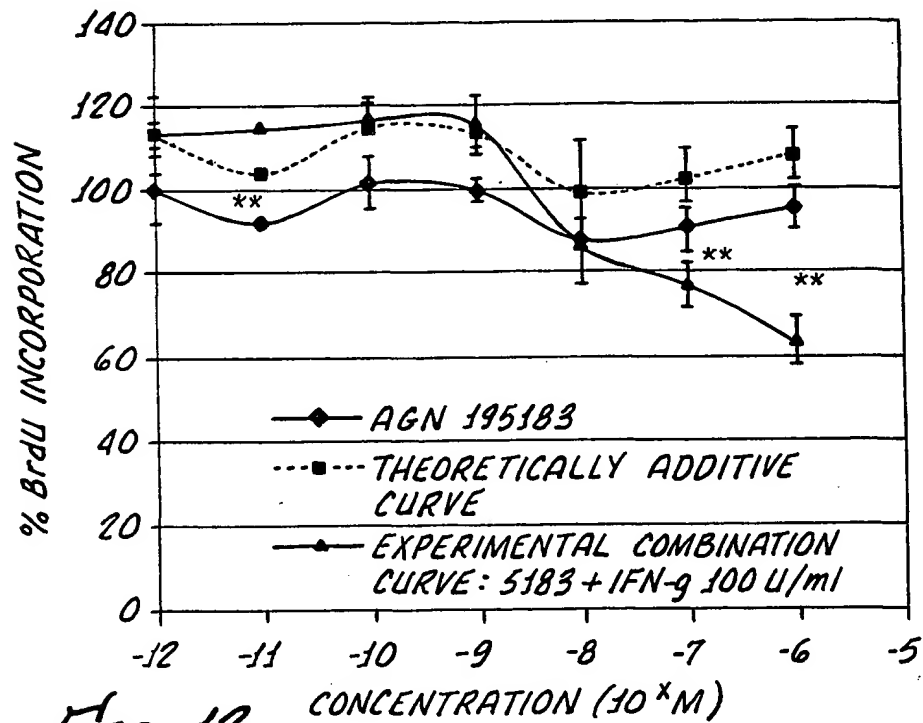


FIG. 12.

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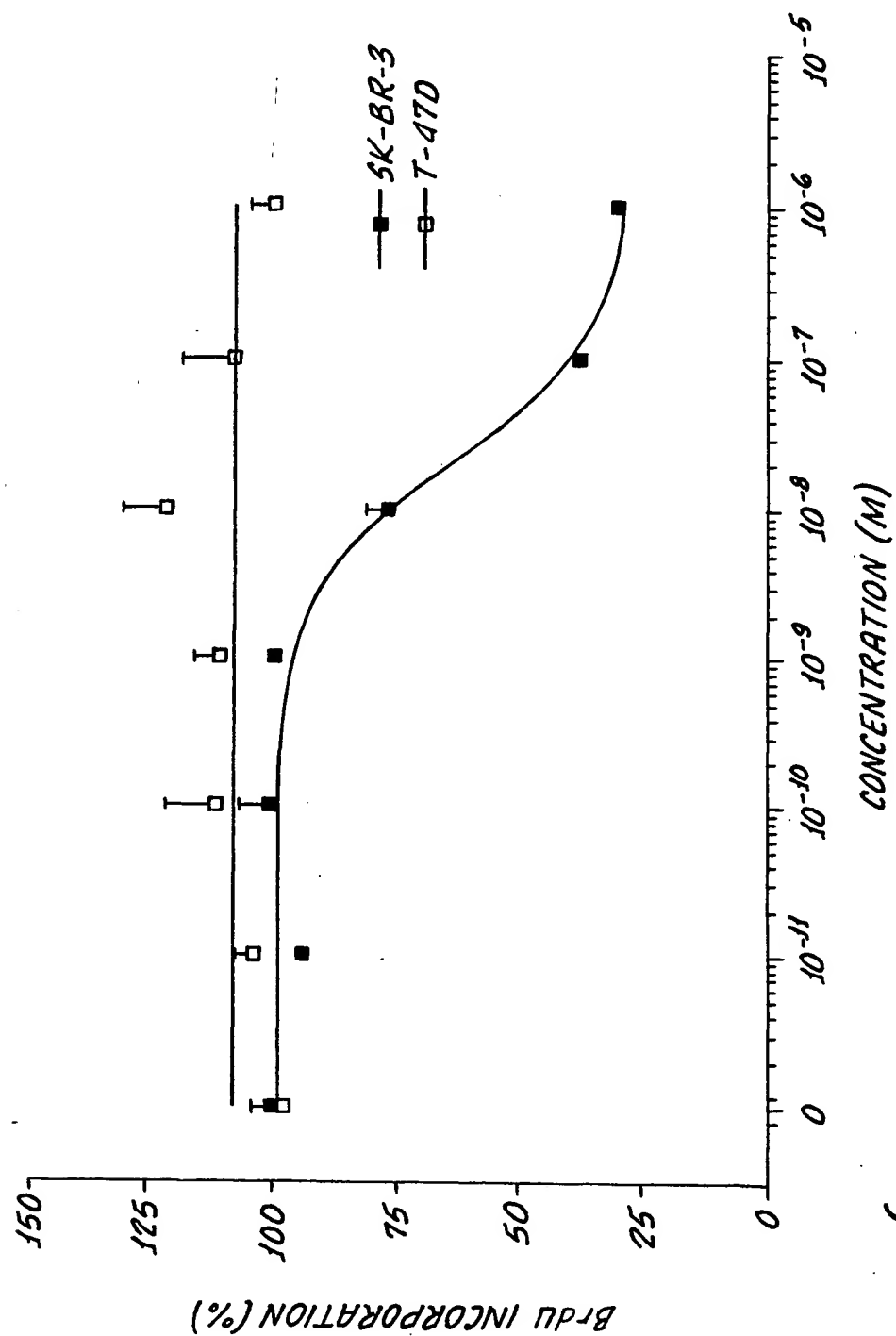


FIG. 13.

INTERNATIONAL SEARCH REPORT

Int. Patent Application No

PCT/US 01/10410

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C233/81 A61K31/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 19052 A (ALLERGAN INC) 29 May 1997 (1997-05-29) page 15 -page 17; claims 1,16 & US 5 856 490 A 5 January 1999 (1999-01-05) cited in the application ---	1-6,13, 14
X	WO 97 24116 A (ALLERGAN INC) 10 July 1997 (1997-07-10) page 59; claims 1-5 & US 5 965 606 A 12 October 1999 (1999-10-12) cited in the application ---	1-6,13, 14
-/--		



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

25 June 2001

Date of mailing of the international search report

10/07/2001

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/10410

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TENG M ET AL: "IDENTIFICATION OF A RETINOIC ACID RECEPTOR ALPHA SUBTYPE SPECIFIC AGONIST" JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 39, no. 16, 2 August 1996 (1996-08-02), pages 3035-3038, XP000652115 ISSN: 0022-2623 the whole document	1,4
X	MATTHIEU SCHAPIRA ET AL.: "Rational discovery of novel nuclear hormone receptor antagonists" PROC. NATL. ACAD. SCI. , vol. 97, no. 3, 1 February 2000 (2000-02-01), pages 1008-1013, XP002170486 abstract; table 1	1,4
A	WO 93 11755 A (BAYLOR COLLEGE MEDICINE ;LIGAND PHARM INC (US); SALK INST FOR BIOL) 24 June 1993 (1993-06-24) cited in the application abstract; claims 1-28	1,13,14
A	FANJUL ET AL.: "Potential role for retinoic acid receptor-gamma in the inhibition of breast cancer cells by selective retinoids and interferons." CANCER RESEARCH., vol. 56, 1996, pages 1571-1577, XP001009864 AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD., US ISSN: 0008-5472 cited in the application the whole document	1,4,7-16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/US 01/10410

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9719052 A	29-05-1997	US 5675024 A	07-10-1997
		AT 200074 T	15-04-2001
		AU 702499 B	25-02-1999
		AU 1056097 A	11-06-1997
		BR 9611758 A	30-03-1999
		CA 2238274 A	29-05-1997
		CN 1214675 A	21-04-1999
		DE 69612303 D	03-05-2001
		EP 0876330 A	11-11-1998
		JP 2000500499 T	18-01-2000
		NZ 323581 A	29-07-1999
		PL 327457 A	07-12-1998
		US 6034244 A	07-03-2000
		US 6124455 A	26-09-2000
		US 5856490 A	05-01-1999
WO 9724116 A	10-07-1997	US 5965606 A	12-10-1999
		AU 710483 B	23-09-1999
		AU 1345097 A	28-07-1997
		BR 9612318 A	28-12-1999
		CA 2241758 A	10-07-1997
		CN 1211180 A	17-03-1999
		CZ 9802069 A	14-04-1999
		EP 0869782 A	14-10-1998
		HU 0001643 A	28-05-2001
		JP 2000502707 T	07-03-2000
		PL 327627 A	21-12-1998
WO 9311755 A	24-06-1993	AT 163544 T	15-03-1998
		AU 679171 B	26-06-1997
		AU 3421893 A	19-07-1993
		CA 2123223 A	24-06-1993
		DE 69224660 D	09-04-1998
		DE 69224660 T	16-07-1998
		DE 617614 T	08-06-1995
		DK 617614 T	28-09-1998
		EP 0617614 A	05-10-1994
		EP 0807624 A	19-11-1997
		ES 2066750 T	16-03-1995
		JP 8502238 T	12-03-1996
		US 5968989 A	19-10-1999
		US 5932622 A	03-08-1999